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Research Article

PREPARATION AND EVALUATION OF THERMOREVERSIBLE FORMULATIONS OF FLUNARIZINE HYDROCHLORIDE FOR NASAL DELIVERY

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ABSTRACT

The purpose of the present investigation was to prepare and evaluate thermo reversible formulations of flunarizine hydrochloride for improved drug residence time in the nasal cavity. The formulations so prepared were in the liquid state at 4°C while turned into a gel at the temperature of the nasal cavity. Poloxamer 407 was used as the polymer which exhibited the phase transition behavior. Inclusion complexes using β-CD were prepared for increasing the solubility of flunarizine in nasal secretions. The prepared formulations were characterized for drug loading, content uniformity, in-vitro drug diffusion, bioadhesion strength, gel strength, viscosity, inflection and gelation point. The formulations exhibited drastic increase in the viscosity at the temperature of 37°C indicating their possible use as in-situ gelling systems. Out of all the formulations studied the β-CD formulations shows the fastest release, possibly due to increase in the solubility and dissolution rate of flunarizine Hydrochloride.

Keywords: Flunarizine HCl, Gels, Nasal, Poloxamer 127, Thermo reversible.

INTRODUCTION

Drugs have been administrated nasally for therapeutic and recreational purpose since ancient times. Psychotropic drugs and hallucinogens were snuffed for these purposes by the Indian of South America, and this practice is currently widespread among abusers of cocaine and heroine[1]. The interest in and importance of the systemic effects of the drugs administrated via the nasal route have expanded over recent decades. Nasal administration offers an interesting alternative for achieving systemic drug effects to the parenteral route, which can be inconvenient for oral administration, which can result in unacceptably low bioavailabilities[2]. The nasal epithelium is a highly permeable monolayer, the sub mucosa is highly vascularised with large and fenestrated capillaries facilitating rapid absorption. Moreover, direct systemic absorption avoids hepatic first-pass metabolism, gut wall metabolism and destruction in gastrointestinal tract [3].Owing to these merits; various nasal drug delivery systems are available for user-friendly noninvasive painless application.

In spite of the advantages cited above, the nasal route of drug delivery is associated with several limitations such as risk of local side effects and irreversible damage of the cilia on the nasal mucosa, both from the drug and from the excipients[4]. The absorption enhancers used may disrupt and even dissolve the nasal membrane in high concentration. Also, the untoward immunogenic effects might arise with the route. The most important limitation is the mechanical loss of the dosage form into the other parts of the respiratory tract like lungs due to high mucociliary clearance[5] which results in low bioavailability due to short residence time of the drug at the site of absorption. The various formulations administered by the nasal route include the nasal powders[6], drops and spray[7, 8]. Apart from these, in order to increase the residence times of the drug in the nasal cavity, Bioadhesive formulations[9], microspheres[10] and gels[11] are used.

Gels are the polymeric network which increases the contact time of drug at the administered site, hence increasing the absorption of drug, due to its mucoadhesive property. However, it shows less patient compliance owing to its semi solid nature and difficulty in application. Hence the patient compliance can be improved be designing the formulation such that it is liquid during application but turns into a gel when comes in contact with the environment of the nasal cavity. Thermo reversible gels can be formulated using environmentally responsive polymer such as poloxamers[12]. Lutrol F grades are block copolymers referred to as poloxamers, consisting of Polyoxyethylene (POE) and polyoxypropylene (POP) units. Higher molecular weight poloxamer has the ability to form thermo reversible gels. In particular poloxamer 407 (Lutrol F 127) has been used in number of applications including nasal drug delivery, where the increase in viscosity at body temperature, increases the residence time of the drug in the nose[13]. The drug release is dependant on the dissolution of the gel, which is a zero order process. As pluronic F-127 is fulfilling all the properties required to form successful nasal formulation, with increased contact time, it was taken as the polymer of choice to prepare thermoreversible gel. Flunarizine Hydrochloride is an anti-migraine drug presently available in the form of tablets and capsules. It undergoes extensive first pass effect[14] leading to low and variable bioavailability. These demerits of flunarizine Hydrochloride can be avoided if given by nasal route in the form of thermo reversible gels.

MATERIALS

Flunarizine HCL was a gift sample from FDC Limited (Roha). Pluronic F-127 was procured from ICPA Health Products Ltd, Ankleshwar, Gujarat. β-Cyclodextrin was supplied by Ajanta pharma, Mumbai, polyethylene glycol 4000 and 6000 from Qualigens Fine Chemicals, Mumbai, Propylene Glycol, Benzalkonium Chloride and Sodium bicarbonate from Loba - Chemie Pvt. Ltd, Mumbai. All other chemical and reagent used in the study were of analytical grade.

METHODS

Determination of λmax of Flunarizine Hydrochloride

A stock solution of 100µg/ml of flunarizine hydrochloride was prepared by dissolving 10 mg in 100 ml of distilled water. The resulting solution was scanned between 200 nm to 400 nm using UV-visible spectrophotometer UV 1700, Shimadzu, Pharmaspec, Japan.

Determination of gelation temperature of pluronic F-127

Solutions of pluronic F-127 was prepared according to the cold method described by Schmalka[15]. Required quantity of polymer was weighed and added to water at about 4°C and stirred. It was then refrigerated overnight to ensure complete dissolution of the polymer in water resulting in clear transparent solution at 4°C. Five different concentrations viz. 20, 22, 24, 26 and 28% w/w of pluronic F127 were employed to determine the gelation temperature. 10 ml of each solution was taken in a test tube and placed in a water bath, whose temperature was increased gradually. For each solution, the temperature at which a solid gel formed was measured using a calibrated thermometer. This temperature represented the gelation temperature

Effect of excipients on gelation temperature of Pluronic F127

To study the effect of excipients on the gelation temperature of pluronic F 127, 20%w/w of pluronic F 127 solution was used. The

effect of each excipient on the gelation temperature was studied by adding each excipient separately to pluronic solution and measuring the gelation temperature as discussed earlier. The excipients studied were Polyethylene Glycol 6000 (2%) PEG 4000 (2%) Propylene glycol and Benzalkonium chloride (0.02%). The effect of other excipients was already known from the literature available[16]. The effect of drug on gelation temperature was also studied.

Preparation of formulations

Three different types of formulations were prepared viz. formulations containing ethanol, simple formulations and formulations containing β-CD. The composition of the various formulations is shown in Table 1. Accurately weighed quantity of Pluronic F127, Polyethylene glycol 4000 and propylene glycol were dissolved in distilled water at 4°C. The mixture was allowed to stand at 4°C for 24 hours until a clear solution was obtained. (Solution A).

For ethanol containing formulations, 10 mg of flunarizine was dissolved in ethanol and added in the solution A. Simple formulations were prepared by adding 10 mg of flunarizine hydrochloride directly in the solution A and stirring it till the drug dissolves. The pH of this formulation was below the pH range (4.5-6.5). So pH was adjusted with the help of sodium bicarbonate (5%) solution. The amount of sodium bicarbonate (5%) required for pH adjustment was optimized, so that there is no precipitation of drug.

For formulations containing β-CD, 10 mg of flunarizine HCL was dissolved in ethanol and β-CD was dissolved in water, and the solutions were mixed and stirred together at room temperature for 24hrs. The mixture was then centrifuged, frozen & lyophilized. This lyophilized mixture was then added to solution A.

Table 1: Composition of different formulations prepared

Formulation	PEG-4000	PG	Pluronic	Ethanol	β-CD
A	1.5%	1.0%	19.5%	4%	-
B	2.0%	1.0%	19.5%	4%	-
C	1.5%	2.0%	19.5%	4%	-
D	2.0%	2.0%	19.5%	4%	-
E	1.5%	1.0%	18.5%	-	-
F	1.5%	2.0%	18.5%	-	-
G	2.0%	1.0%	18.5%	-	-
H	2.0%	2.0%	18.5%	-	-
I	1.5%	1.0%	17.5%	-	1.5%
J	1.5%	2.0%	17.5%	-	1.5%
K	2.0%	1.0%	17.5%	-	1.5%
L	2.0%	2.0%	17.5%	-	1.5%

CHARACTERIZATION OF THE PREPARED FORMULATIONS

Inflection point

As the temperature of the formulations was increased from 4°C, a point reached where drastic change in the viscosity was observed, that point was considered as Inflection point.

Gelation point

It is temperature at which the liquid phase makes a transition to gel. A gelation temperature range suitable for thermo reversible nasal gel would be 30-36°C. Gelation point was considered as the temperature where formulations would not flow when test tubes were tilted to 90° angle, as the temperature was gradually increased.

pH of the gels.

The pH of each batch was measured using Elico pH meter which was calibrated using buffers of pH 4 and pH 8 before the measurements.

Content uniformity

Weighed amount of the formulation was dissolved in distilled water and after suitable dilution; the absorbance was measured using UV-VIS spectrophotometer at 291.5 nm. The amount of the drug present in the formulation was calculated by measuring the absorbance of a standard solution of known concentration of flunarizine hydrochloride prepared in distilled water

Rheological studies

Viscosity of the prepared formulations was measured by using Brookfield synchro Viscometer. The gel under study was placed in the small sample holder and the spindle was lowered perpendicularly into it. The spindle was rotated at varying speeds and the suitable speed was selected. Temperature was increased initially above 40°C and then the viscosity was measured as the system was allowed to cool gradually.

Measurement of gel strength[12]

Formulated gels were placed in the test tubes and gelled in a thermostat at 37°C. The apparatus for measuring gel strength (Weight: 27gm.) was then placed onto the Poloxamer gel (Figure 1). The time taken by the apparatus to sink to a dept of 5 cm through the prepared gel was measured for each formulation.

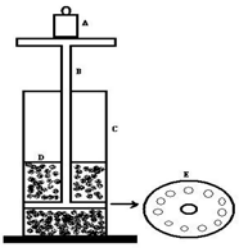

Fig. 1: Determination of gel strength

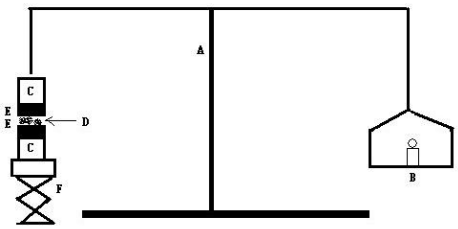

Fig .2: determination of bioadhesive strength.

Determination of bioadhesive force[12]

The bioadhesive force of all the batches was determined by using measuring device in Figure 2. In brief, a section of nasal mucosa was cut from the sheep's nasal cavity and instantly secured with mucosal side out onto each glass vial (C) using rubber band. The vials with the nasal mucosa were stored at 37°C for 5 min. Next, one vial with a section of mucosa (E) was connected to the balance (A) and the other vial were placed on a height – adjustable pan (F). Formulation (D) was

added onto the nasal mucosa on the other vial, one by one. Then, the height of vial was adjusted so that the gel could be placed between the mucosal tissues of both vials. The weight (B) was kept raised until two vials were attached. Bioadhesive force, the detachment stress, was determined from the minimum weight that detached two vials. The nasal mucosa was changed for each measurement.

In vitro nasal diffusion cell [17, 18]

The nasal diffusion cell was fabricated in glass. The water-jacketed recipient chamber has total capacity of 60ml and a flanged top of about 3mm; the lid has 3 openings, each for sampling, thermometer, and donor tube chamber. The 10cm long donor chamber tube has internal diameter of 1.13cm. The nasal mucosa of sheep was separated from sub layer bony tissues and stored in distilled water containing few drops of gentamycin sulfate injection. After the complete removal of blood from mucosal surface, it was attached to donor chamber tube. The receptor chamber was filled with 60 ml distilled water and agitated continuously using a magnetic stirrer. The cell was equilibrated at 37+2 ^{0}C. The donor chamber tube was lowered to just touch the diffusion medium in recipient chamber. The drug formulations were placed on the dorsal surface of nasal mucosa.

RESULTS AND DISCUSSION

Flunarizine Hydrochloride exhibited λmax at 291.5 nm. Linearity was observed in the range of, 20 to 100 µg/ml with the r^2 value of 0.999. The preliminary studies indicated that the minimum concentration of PF-127 that formed gel below 35°C was 18.66% w/w. The gelation temperature of different concentration of pluronic F-127 is shown in Table 2.

Table 2: Effect of concentration of pluronic F-127 on gelation temperature

Concentration (%w/w)	Gelation Temperature (°C)
20	24.5±0.4
22	23.5±0.2
24	22.2±0.3
26	20.5±0.5
28	19.3±0.1

As shown in the table 2, it was observed that as the concentration of pluronic F-127 increased, the gelation temperature decreased. As the concentration of pluronic F127 increases, there is micelle formation, followed by micellar aggregation. The gel phase can only occur when the concentration is above the micellar concentration[19].

When the material is in cold water, hydrogen bonding between POP chains and water keeps the hydrophobic portions of the pluronic separate. When the temperature is increased, the hydrogen bonding is disrupted, and hydrophobic interactions cause a gel to be formed. Therefore, the gelling properties of the poloxamers are dependent on percentage of hydrophobic portion. As the concentration of pluronic F127 increases, the hydrophobic portion also increases resulting in formation of gel at lower temperature.

It was previously reported that addition of drug alone to PF-127 solutions in clinically useful levels would result in loss of required gelation properties. Incorporation of PEG maintains the gelation properties while increase in drug loading[20]. Propylene glycol was added for its humectant's properties and Benzalkonium chloride was added as a preservative as it has been reported to be well tolerated in human nasal mucosa[21]. The effect of different excipients on the gelation temperature of pluronic F127 was studied and it was found that PEG 6000, Propylene glycol and flunarizine HCl increased the gelation temperature whereas PEG 4000 decreased the gelation temperature. PEG 4000 was chosen for further study as it decreased the gelation temperature leading to reduced requirement of pluronic F127.

The gelation temperature has been considered to be suitable if it is in range of 25°C to 37°C[13]. If gelation temperature of thermo reversible formulation is lower than 25°C, a gel might be formed at room temperature leading to difficulty in manufacturing, handling and administering. If the gelation temperature is higher than 37°C, it would not form gel at the temperature of the nasal cavity resulting in rapid nasal clearance of administered drug. Due to this reasons, the formulations were prepared such that the gelation temperature is maintained between 25°C -37°C.

Three different types of formulations were prepared: Formulations containing ethanol, simple formulations and formulations of β-CD complexed drug. Ethanol was added in order to increase the drug loading. In order to achieve the desired gelation temperature after adding the excipients, 19.5%w/w of pluroinic F 127 was required. The minimum ethanol concentration required for optimum drug loading was found to be 4%.

It was observed that in case of simple formulations, when flunarizine was added along with the other excipients in the pluronic solution at 4°C, it precipitated after 24 hrs, resulting in poor drug loading. In order to increase the drug loading, initially only the excipients were added in the pluronic solution at 4°C and after 24 hrs, drug was added with stirring to this clear solution. For simple formulations, the pluronic concentration required to achieve the desired gelation temperature was 18.5%w/w.

In the third type of formulation containing β CD-Drug complex, only 17.5% w/w pluronic F-127 was required for attaining the desired gelation temperature.

Inflection Point, Gelation Point, pH and Viscosity of the Formulations

The inflection point, gelation temperature, pH and viscosity of the various formulations are shown in table 3. It can be seen from the table 3, that there is definite relation of the inflection and gelation point with viscosity. The formulations which exhibited minimum inflection and gelation point had maximum viscosity at 37°C.

Table 3: Inflection Point, Gelation Point, pH and Viscosity of Formulations

Formulation	Inflection Point (°C)	Gelation Temp. (°C)	pH	Viscosity at 37^{0}C cp(x10^3)
A	30.0	32.0	4.57	720
B	32.5	33.5	4.78	475
C	33.5	35.0	5.04	115
D	32.5	33.5	5.02	524
E	34.5	36.0	4.82	496
F	32.5	34.0	4.52	356
G	33.0	35.0	5.14	332
H	29.0	31.0	5.24	790
I	29.0	32.0	4.63	227
J	30.5	34.0	4.99	155
K	31.0	35.0	4.52	104
L	33.5	37.0	4.59	175

Out of all the formulations, the simple formulation H had minimum inflection and gelation point and maximum viscosity and hence is expected to have prolonged residence time in the nasal cavity. pH is a very important factor for nasal formulations[22]. Preparations within

a pH range of 3-10 causes the minimal release of the biochemical makers, where as solutions of pH above 10 or less than 3 causes significant membrane damage and intracellular enzyme release.

The narrow range of pH for nasal formulation is 4.5-6.5. Alkaline pH inactivates the lysozyme secreted by nasal cells, hence makes the nasal tissue susceptible to microbial infection. Lower pH acts as hypertonic solutions, causing the shrinkage of epithelial cells and also inhibits ciliary activity. All the batches were found to have satisfactory pH. So no irritation to nasal mucosa due to pH is expected.

Content Uniformity, Bioadhesive Force and Gel Strength

The content uniformity, bioadhesive force and gel strength of the prepared batches is shown in table 4. All the batches formulated were found to have satisfactory content uniformity. The Bioadhesive force indicates the strength of the bioadhesion between the gel and the nasal mucosa. It was found that the formulations having high Bioadhesive force had high gel strength also. Out of all the formulations, formulation H showed the highest Bioadhesive force and gel strength indicating a significant improvement in the drug residence time. The results are also in concurrence with the results of inflection, gelling point and viscosity.

Table 4: Content Uniformity, Bioadhesive Force and Gel Strength of the Formulations

Formulation	Content	Bioadhesive Force (gms)	Gel Strength (sec)
A	99.3 %	10.499	117
B	99.4 %	6.889	108
C	99.4 %	4.765	69
D	99.3 %	10.352	116
E	100.1 %	9.342	116
F	99.3 %	11.234	102
G	100.1 %	12.498	102
H	99.2 %	13.805	151
I	102.4 %	13.431	102
J	101.5 %	11.021	98
K	100.2 %	11.310	96
L	100.3%	09.216	85

Diffusion Studies

The drug diffusion from the prepared formulations through the nasal mucosa is shown in figures 3a, 3b and 3c. The drug diffusion from ethanol containing formulations is shown in figure 3a. As it can be seen from the table 5, formulation D shows the fastest diffusion and approximately 50% of drug was diffused within 2 hrs. Out of all the prepared formulations, β-CD formulations exhibited highest extent and rate of drug diffusion, possibly due to increased solubility and dissolution rate of the drug. Thus the β-CD formulations have an advantage of showing fast drug diffusion in addition to turning into a gel when in contact with the nasal mucosa. These formulations thus seem to be most promising as nasal drug delivery systems.

The drug diffusion from the β-CD containing formulations is shown in figure 3c Amongst the β-CD formulations, formulation J showed the highest rate of drug diffusion and within 15 minutes, 50% of the drug is diffused.

Table 5: Time for 50% and 100% diffusion of drug from the Formulations

Formulation	Time for approx. 50% Diffusion (hrs)	% Diffusion	Time for approx. 100% Diffusion (hrs)	% Diffusion
A	3.0	49.13	5.0	95.38
B	3.0	53.10	4.3	94.18
C	3.0	48.82	4.3	94.18
D	2.0	50.63	4.3	92.92
E	2.0	51.50	7.0	98.55
F	2.0	48.92	7.0	98.43
G	2.0	53.87	7.0	96.42
H	1.0	48.50	6.0	93.09
I	0.5	50.57	4.0	101.83
J	0.25	52.28	5.0	100.74
K	1.0	48.50	5.0	99.67
L	0.5	48.12	5.0	99.92

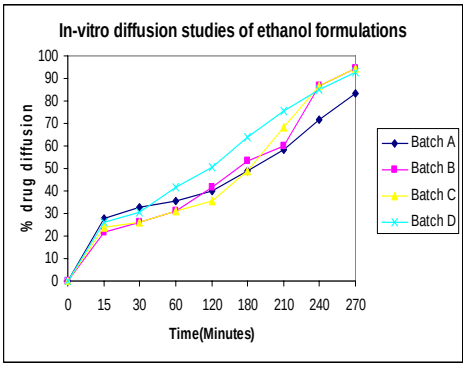

Fig. 3 a: *In vitro* Diffusion Study of ethanol containing formulations

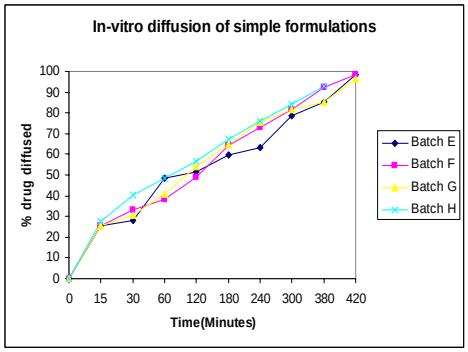

Fig. 3 b: *In vitro* Diffusion Study of simple formulations

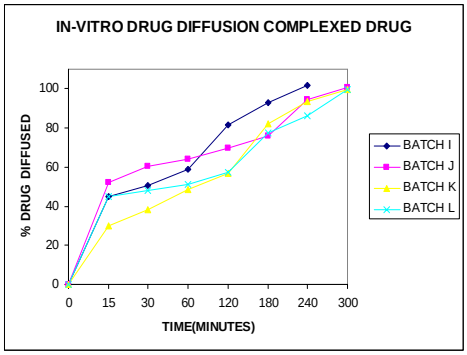

Fig. 3 c: *In vitro* Diffusion Study of β-CD containing formulations

CONCLUSION

The present investigation indicates that thermoreversible formulations of flunarizine HCl using pluronic F127 seems to be promising nasal drug delivery system. There is drastic increase in the viscosity of the formulation at the temperature of the nasal cavity indicating the possibility of the in-situ gelling. There was no significant difference with respect to bioadhesive strength and gel strength between the different formulations. However, when the drug release of the various formulations were compared, it was found that formulations containing β-CD showed significantly faster drug release compared to the other formulations. Hence, as desirable the β-CD formulations are expected to improve the drug residence time as well as rate of absorption and hence are expected to improve the bioavailability of the drug during in-vivo studies. The present study indicates that much more work can be done on the Nasal formulation of Flunarizine HCl. The concentration of drug in Cerebrospinal fluid can be measured by in vivo studies, so as to determine the direct entry of drug into the brain through olfactory route.

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